Supporting Information

SI text

Additional differentially regulated genes identified with RNA-sequencing. Of the remaining 25 differentially regulated genes, 15 up-regulated genes are largely uncharacterized but found clustered together on the chromosome in four groups, labeled unknown group 1-4 (Table S1), some of which are predicted to be phage-related based on their sequence similarity to known phage genes. The remaining five up-regulated genes include, PA14_52300, which is predicted to be SOS-regulated due to the proximity to a LexA box in the upstream regulatory region, *nirS* a nitrite reductase, PA14_52380, which encodes cytochrome b561, and two uncharacterized genes, PA14_46670 and PA14_52440.

Strain construction. All strains and plasmid constructs were generated using allelic exchange and *Saccharomyces cerevisiae* recombineering techniques described previously (1, 2), including the construction of the DMS3-42 protospacer chromosomal knock-on strain (PS-CKOn) in which the DMS3-42 protospacer and PAM was inserted using allelic exchange at the *att*::Tn7 site. It is important to note that while the protospacer and PAM were inserted at the *att*::Tn7 site, the previously reported Tn7 insertion system (3) was not used to insert these sequences. Thus the entire insertion is just the 32 nt protospacer plus 5 flanking nt on each side (including the PAM) for a total insertion of 42 nt.

For construction of the A19C and C18G PS-CKOn plasmid a modified point mutagenesis technique was used. Briefly, forward and reverse primers containing either the A19C or C18G mutant were used in separately PCR reactions to amplify sequences from the parent DMS3-42 PS-CKOn vector. After four cycles of amplification, the forward and reverse reactions were combined and continued for 18 cycles. Any remaining parental vector was digested using the

DpnI restriction enzyme for four hours at 37^{o}C, and the resulting products were transformed into S17 *E. coli*. These constructs were verified by sequencing.

Congo red assay: The indicated strains were grown overnight in 5 mL LB with shaking at 37 degrees C. Using these overnight cultures, 2.5 µL of each indicated strain was spotted on M63 1% agar supplemented with 5% casamino acids, 2% glucose, 1mM $MgSO_4$, 40 µg/mL Congo red, and 20 µg/mL Coomassie brilliant blue, followed by incubation at 37 degrees C for 16 hours and subsequent incubation at room temperature for 48 hours.

Growth Curves: Strains were grown overnight in 5 mL LB with shaking at 37 degrees C and normalized to OD_{600} of 1.0. Cells were then diluted 1:1000 in 5 mL fresh LB and incubated for 8 hours. At every hour, OD_{600} was measured with a Spectronic 20D+ spectrophotometer.

Swarm assay. Indicated strains were grown overnight in LB with shaking at 37 degrees C and 2.5 µL of each strain was inoculated in the center of a 0.5% soft agar M8 plate supplemented with MgSO4 (1mM), glucose (0.2%), and casamino acids (CAA; 0.5%), as described (4). The plates were incubated for 16 hours at 37 degrees C and subsequently imaged.

Fluorescence microscopy viability assay. To visualize adhered biofilm cells, the cover slip was washed twice in Tris-buffered saline (TBS) and stained with Molecular Probes® Live/Dead *Bac*Light™ as per manufacturers instructions. For the planktonic population, bacteria were directly harvested from the medium surrounding the coverslip, washed once in TBS, and stained with *Bac*Light™ as per manufacturers instructions. The stained coverslip, or 5µL of the stained planktonic cells prepared as a wet mount, were visualized with a Nikon eclipse Ti inverted microscope. The quantification of live versus dead cells in the biofilm was performed as previously reported (5), with slight modifications. Briefly, fluorescent images were acquired from random fields at the air-liquid interface of the biofilm and random fields of planktonic cells from the wet mounts. NIS elements AR version 3.2 software was used to measure mean fluorescent intensity (MFI) for both Syto® green and propidium iodide in each image, which was averaged

50 over at least 10 images. For both biofilm and planktonic populations, the data presented are an
51 average from at least 3 biological replicates.
52 **Identification of differentially expressed genes from RNA-seq data.** After read
53 mapping with CLC Genomics Workbench, the number of unique counts for each gene was used
54 as input for differential gene expression analysis with edgeR (6) using the R software package
55 Version 3.0.1. Samples were normalized with edgeR using the trimmed mean of the $\log_2$ counts
56 (TMM) method. Normalization factors and pseudocounts were calculated for each sample to
57 account for differences in library size. Differentially expressed genes were identified with an
58 exact test for the negative binomial distribution (7-9). Since the RNA-Seq experiment did not
59 include replicate samples, the common dispersion parameter was estimated to be 0.05 based
60 on a previous RNA-Seq experiment with 3 replicates of wild type *P. aeruginosa* PA14
61 (unpublished observation).
62 **Pyocin killing assay**: For each indicated strain, cells were grown in LB for 16 hours at
63 37 degrees C with shaking and normalized to OD_{600} of 3.0, then 1 mL of each sample was filter-
64 sterilized using a Millex® GV 0.22 µM syringe filter (Millipore). The filtrate was serially diluted
65 (1:10) in LB. A lawn of pyocin-sensitive *P. aeruginosa* strain PAK was generated by adding 100
66 µL of LB-grown *P. aeruginosa* PAK (OD_{600} = 3.0) to 3 mL of molten top agar (0.8%), and poured
67 over a pre-warmed LB agar plate. 5 µL of the indicated diluted filtrate was spotted onto the *P.*
68 *aeruginosa* PAK lawn and incubated overnight at 37 degrees C. Killing was scored by the
69 appearance of a clear plaque in the lawn of the *P. aeruginosa* PAK cells.
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Literature Cited

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